Section II (Remarks)

A. Summary of Amendment to the Claims

By the present Amendment, claim 1 has been amended. No new matter within the meaning of 35 U.S.C. §132(a) has been introduced by the foregoing amendment. Specifically, the amendment to claim 1 is supported by claim 3, as originally filed.

The amendments made herein are fully consistent with and supported by the originally-filed disclosure of this application.

B. Claim Rejections Under 35 U.S.C. §103

In the Office Action mailed March 19, 2010, the examiner maintained the rejections under 35 U.S.C. §103. Specifically the examiner maintained the rejection of claims 1-5, 7, 11, and 12 under 35 U.S.C. §103(a) as obvious over Francisco, et al., *Proc. Natl. Acad. Sci. USA*, 90 10444-10448, 1993 (hereinafter "Francisco et al.") or Charbit et al., *Gene*, 70, 1, 181-189, 1988 (hereinafter "Charbit et al.") in view of Lee et al, *Trends in Biotechnol.*, 21, 1, 45-52 (hereinafter "Lee et al.") and Christalli et al., *Arch. Biochem. Biophys.* 377, 2, 324-333, 2000 (hereinafter "Christalli et al.").

Additionally the examiner maintained the rejection of claims 1-7, 11, and 12 under 35 U.S.C. §103(a) as obvious over Francisco, et al. or Charbit et al. in view of Lee and Christalli and further in view of Park et al., *FEMS Microbiol. Lett.* 214, 217, 2002 (hereinafter "Park et al.") or DeBoer et al, *Proc. Natl. Acad. Sci,* USA 80, 21-25, 1983 (Hereinafter "DeBoer et al.").

The examiner also maintained the rejection of claims 1-5, and 7-12 under 35 U.S.C. §103(a) as obvious over Francisco, et al. or Charbit et al. in view of Lee and Christalli and further in view of U.S. Patent No. 5,508,192 (hereinafter "Georgiou et al.").

Furthermore the examiner maintained the rejection of claims 1-5, 7, 11, 12, and 13 under 35 U.S.C. §103(a) as obvious over Francisco, et al. or Charbit et al. in view of Lee and Christalli and further in view of U.S. Patent No. 6,071,725 (hereinafter "Pan et al.").

Applicants respectfully maintain the traversal of all four of the above rejections. Initially it is noted that by the Response mailed September 4, 2009, claims 2 and 3 were cancelled. Accordingly, the examiner's rejections are addressed below as applicable to claims 1 and 4-13.

All of the rejections are based on the primary references Francisco, et al. or Charbit et al. Additionally, all of the rejections further rely on secondary references Lee et al. and Christalli et al. The discussion of these four references, as provided in the Response submitted September 9, 2009, is incorporated herein by reference, as if restated herein and is applicable to all of the stated rejections.

By the present Response claim 1 has been amended to recite:

> "1. A vector for expressing a target protein on the surface of cells, the vector comprising a gene recombinant including a *fadL* gene encoding an *E. coli* outer membrane protein (FadL) in which the C-terminal end of the FadL protein is truncated, an antibiotic-resistant gene, a promoter, and a gene encoding a target protein, in which the gene recombinant is constructed such that if the target protein-encoding gene is expressed in a host cell, it is expressed on the surface of the cell in a form fused with the FadL protein, wherein the target protein-encoding gene is positioned after the *fadL* gene encoding the truncated protein."

By such amendment it has been made clear that applicant's claimed vector comprises a *fadL* gene encoding a truncated protein and that the target protein-encoding gene is positioned after the *fadL* encoding the truncated protein.

By applicants' invention, the surface display outer membrane FadL is used as a surface anchoring motif for a target protein or peptide. In the production of a cell with the target protein expressed on the surface, applicants determined the optimal fusion point of FadL with target protein to express target protein on cell surface effectively among various possible fusion points of FadL.

In one attempt, applicants removed the residues of FadL corresponding to 2nd of the 10 loops of FadL, however, the expression efficiency was not well achieved. After investigating various locations on *fadL* for truncation and attachment of the target protein gene, the inventive location was determined. As recited in the claims, the portion of the gene encoding the C-terminus of FadL is removed and the gene for the target protein is attached thereto. Accordingly, the *fadL* gene in the claimed vector is not a complete *fadL* gene, but is a truncated gene, with the C-terminus removed.

It is well established that a showing of obviousness must include a reasonable expectation of success. ("The prior art can be modified or combined to reject claims as *prima facie* obvious as long as there is a reasonable expectation of success. *In re Merck & Co., Inc.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986)" MPEP §2143.02). None of the cited combination of references provides a vector coding for a truncated *fadL* gene with a gene for a target protein attached thereto with a reasonable expectation of success of successful expression of the target protein on a cell surface.

Applicants' prior arguments acknowledged that Francisco et al. and Charbit et al. each "disclose a vector for expressing a target protein on the surface of cells…" (Office Action mailed March 19, 2010, p. 2-3) and that Lee et al. and Christalli et al. "disclose the outer member protein FadL and its structure…" (Christalli et al.) and "disclos[ing] the general method of displaying proteins of choice on the surface of microbes using fusions such as C-terminal, N-terminal or sandwich type fusions of the protein of choice with 'carrier proteins', which are outer membrane proteins…" (Office Action mailed March 4, 2009, p. 3.)

However, it was applicants' position that one of skill in the art would not have had a reasonable expectation of success in the combination of Francisco et al. or Charbit et al. with Lee et al. and Christalli et al. Specifically, the examiner's attention was directed to the "Background Art" section of the application, where the advantages of Applicants' invention were noted.

In response, the examiner asserted that "the fact that the references cited disclose at least 5 widely diverse outer membrane proteins that are used as surface anchoring motifs…shows that any outer membrane protein, such as fadL, is likely to be a useful outer display protein. There is no reasons [sic] cited to expect that fadL would differ from the known outer display proteins." (Office Action mailed March 19, 2010, p. 7.)

At page 4 of the specification the physical structure of FadL is described, as "…10 external loops providing various points which can be fused, thus it is expected that the FadL protein can increase the possibility of cell surface expression of foreign proteins…" However by applicant's claimed vector, one of those loops has been removed, and the surface expression of the FadL and/or the target protein would not necessarily be expected.

The characteristics of a protein that is capable of co-expression and surface expression of a target protein are varied. Applicants previously cited a 2002 article by Chen and Georgiou (of record):

> "[u]nfortunately, the mechanisms that dictate targeting and insertion of proteins within the outer membrane are not well understood. Moreover, the incorporation of aberrant proteins within the outer membrane can be toxic to the cell." (Emphasis added.)

Based on the above, it is seen that as of 2002 (as of the time of publication of Chen and Georgiou), some desired characteristics of surface anchoring motifs were known, but the mechanism was not fully understood and that additional such motifs still remained to be discovered. Applicants performed experimentation in order to result in the discovery of FadL as an effective surface anchoring motif.

As previously discussed, Lee et al, *Biotech. and Bioeng.*, 90:223, 2005 (included by Supplemental IDS to the Respnse mailed September 4, 2009; hereinafter "Lee et al. 2005") describes a process for a display of lipase on the cell surface of *E. coli* using $OprI^2$ as an anchor. As described in the RESULTS and DISCUSSION sections (right column of page 226 to page 229), the fusion site for cell surface display in $OprI^2$ determines host cell growth. In the 2nd paragraph of the DISCUSSION section, it is described that the authors developed a cell surface display system using the P. *aeruginosa* outer membrane protein $OprI^2$ as an anchoring motif via C-terminal deletion-fusion strategy and this strategy allowed successful display of lipase in an active form on the surface of *E. coli*. In addition, the inventors described how Val188, Ala196, and Arg213 of $OprI^2$ were suggested to be possible fusion sites for the display of small peptides, however, Ala196 and Arg 213 were found to be not suitable for larger protein display because cells did not grow well after over-expression of the respective fusion proteins.

As evidenced by the foregoing discussion, location of a proper fusion site for target protein display on a cell surface is essential for a success of cell surface display, but a vector conformation that will yield the recited cell surface display is not, a prioiri, in any way predictable or even known to be possible. Extensive experimentation has been performed by applicants, resulting in the discovery of the presently claimed vector conformation.

Furthermore, the *fadL* gene in the claimed vector is not a complete *fadL* gene, but is a truncated gene, with the C-terminus removed, where the gene encoding the target protein is then attached.

Either of Francisco, et al. or Charbit et al. further in view of Lee et al. and Christalli et al. fails to provide any derivative basis for the claimed invention. In particular, the cited documents fail to demonstrate that all outer membranes are readily functional as a surface anchoring motif. In fact, as evidenced by Lee et al. 2005, provided above, where various fusion positions were found to be unsuitable for larger protein display, it is clear that the position at which a target protein and an outer membrane protein are fused determines whether target protein has activity or not. The cited references do not provide guidance regarding fusion of an *fadL* gene. Accordingly, one of skill in the art would not have found the claimed vector obvious, in view of the cited references.

None of the additionally cited references: Park et al., DeBoer et al., Georgiou et al., or Pan et al. remedy the deficiencies of the combination of either of Francisco, et al. or Charbit et al. further in view of Lee et al. and Christalli et al.

Based on the foregoing, either of Francisco, et al. or Charbit et al. in view of Lee et al. and Christalli et al. and further in view of any of Park et al., DeBoer et al., Georgiou et al., and Pan et al. fails to provide any logical basis for the vectors, microorganisms or methods recited in claims 1 and 4-13. None of the cited combinations of references renders the claimed invention obvious. Accordingly, withdrawal of the rejections of the claims under 35 U.S.C. § 103(a) as being obvious is respectfully requested.

C. Claim Rejection Under 35 U.S.C. §112

Additionally in the Office Action mailed March 19, 2010, the examine rejected claim 1 under 35 U.S.C. §112, second paragraph, as indefinite for failing to point out and distinctly claim the subject matter which applicant regards as the invention.

Specifically, the examiner rejected the language "the C-terminal end of the fadL gene is truncated at a truncation point" in the claim. The examiner's rejection of the claim was based on the examiner's position that "[i]t is not clear whether it is intended that the complete fadL gene is never present…or whether the complete gene could be present and the 'truncation point' could be after the complete gene…" The examiner's attention is respectfully drawn to Section I above, where claim 1 has been amended. As amended, claim 1 no longer contains the language which is the basis for rejection. The claim does contain the recitation "…in which the C-terminal end of the FadL protein is truncated…" By such amended language, recitation, it is clear that the vector

of to Claim 1 contains a *fadL* gene in which the C-terminal end has been removed, that is, the vector does ***not*** include a "complete" *fadL* gene.

The examiner's attention is respectfully drawn to Examples 1 and 2, where applicants obtained an *E. coli* outer membrane protein (FadL) gene from which the C-terminal end *was removed*. The target protein-encoding gene is linked thereto. Accordingly, it is not possible that the complete *fadL* gene is present, with a "truncation point" after the complete gene, as hypothesized by the examiner.

Claim 1, as pending, is in compliance with the definiteness requirements of 35 U.S.C. §112, second paragraph. Withdrawal of the rejection is respectfully requested.

CONCLUSION

Based on the foregoing, all of applicants' pending claims 1 and 4-13 are patentably distinguished over the art, and in form and condition for allowance. The examiner is requested to favorably consider the foregoing and to responsively issue a Notice of Allowance.

The time for responding to the March 19, 2010 Office Action without extension was set at three months, or June 19, 2010. Applicants hereby request a one month extension of time under 37 CFR § 1.136 to extend the deadline for response to July 19, 2010. Payment of the extension fee of $65.00 specified in 37 C.F.R. § 1.17(a)(1), as applicable to small entity, is being made by on-line credit card authorization at the time of EFS submission of this Response. Should any additional fees be required or an overpayment of fees made, please debit or credit our Deposit Account No. 08-3284, as necessary.

If any issues require further resolution, the examiner is requested to contact the undersigned attorneys at (919) 419-9350 to discuss same.

Respectfully submitted,

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